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**THE LOCATION AND DIFFERENTIATION OF
THE PRESUMPTIVE ECTODERM OF THE
FOREBRAIN AND HYPOPHYSIS AS SHOWN
BY CHORIO-ALLANTOIC GRAFTS**

**A DISSERTATION SUBMITTED TO THE
GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY**

DEPARTMENT OF ZOÖLOGY

1931

By

KATHRYN F. STEIN

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THE LOCATION AND DIFFERENTIATION OF THE PRESUMPTIVE ECTODERM OF THE FOREBRAIN AND HYPOPHYSIS AS SHOWN BY CHORIO-ALLANTOIC GRAFTS¹

(Two plates and three figures)

KATHRYN F. STEIN

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THE original purpose of this investigation was the study of the location and capacity for differentiation of the presumptive hypophysial ectoderm. Data in regard to the capacity for differentiation of the hypophysis and, to some extent, of the ectoderm destined to form it have been reported previously (Stein, 1929). The results obtained at that time showed that the degree of organization was sufficient to cause the formation of a histologically normal hypophysis in chorio-allantoic grafts. This was true in the majority of cases from sixteen somite and older donors and in a few cases from younger donors. The donors used for the previous experiments ranged in age from the headfold stage to 5 days. Comparatively few grafts were made of implants from young donors, headfold and early somite stages. It was therefore thought advisable to extend the work to include these stages, and also the head-process stage, in order to have a more complete story of the location and the capacity for differentiation of the ectoderm which later forms the anterior lobe of the pituitary gland. As in the previous paper, the term "hypophysis" is used to designate the ectodermal structure otherwise known as "Rathke's pocket."

Hunt (1931a) suggested that the medullary plate may depend on the notochord for its differentiation. Certain of the grafts made in the course of this work (of negative value as far as the hypophysis was concerned, since the latter failed to differentiate) seemed, however, to shed some light on the questions of the origin and determina-

¹ It gives me pleasure to express here my appreciation for the helpful suggestions and criticism of Dr. B. H. Willier, who recommended the problem and under whose direction the work was carried on.

tion of the forebrain tissue. Consequently additional grafts were made for the study of these problems.

This paper therefore presents the data obtained from two more or less independent series of experiments. In regard to the forebrain it deals with the location of the tissue giving rise to that structure, with the capacity of that tissue for differentiation when isolated from head-process and from node material, and with the question of the probable time of its determination. The hypophysis and presumptive hypophysial ectoderm are considered in the light of their capacity for differentiation and of the location of the latter.

MATERIAL AND METHODS

Eggs of white Leghorn chickens were used for all the experiments. The method of grafting was that described by Willier (1924) with the exception of slight modifications. A very fine pipette with approximately an inch of the tip bent perpendicularly to the stem was used for transferring the implants to the chorio-allantoic membrane of the 9-day-old host. Solution was first taken into the pipette until the tip remained full even when no pressure was exerted on the bulb. By slight pressure a drop was then ejected and a drop containing the implant taken in. The tissue thus remained at the tip of the pipette during transfer and could be ejected with a minimum amount of fluid by simply touching the tip of the pipette to the chorio-allantoic membrane. Grafts were removed on the sixteenth, seventeenth, or eighteenth days of incubation. All were fixed in Bouin's fluid, sectioned serially at $8\ \mu$, and stained in Heidenhain's iron haematoxylin or, in a few cases, in Ehrlich's haematoxylin and eosin.

For the study of the forebrain the donor embryos used were of primitive-streak, head-process, and early headfold stages. Since number of hours of incubation is not an accurate criterion for judging the actual stage of development of such young embryos, the groupings have been made according to the length of the primitive-streak or head-process. At the time of operation the blastoderm was removed from the yolk and placed in a Petri or Syracuse dish of 0.9 per cent salt solution warmed to 39°C . Measurements were then taken with a Zeiss ocular micrometer which was graded in tenths of a millimeter. Under the Spencer binocular dissecting scope used, with

32 mm. objective and $6\times$ ocular, one unit represents 0.033 mm. Measurements have been expressed in units in the tables, in millimeters in the text of the paper. Knife-bladed needles were used for cutting the blastoderm and after cutting measurements were taken in most cases of the distance of the cut from Hensen's node, the primitive pit, or the head-process, as the case might be. These were approximate measurements, since exact boundaries of these structures are not always clearly distinguishable. There was found to be a great amount of variation among donors in this respect, and for the most part embryos in which structures were not comparatively distinct were discarded. The types of implants made in the three series of experiments on the forebrain are illustrated in Figures 1, 2, and 3 and are described with the results obtained in each case in the section following.

The embryos used for studying the differentiating capacity of the ectoderm destined to form the hypophysis were of head-process, headfold, and early somite stages. The experimental procedure was the same as that described for the experiments on the forebrain. From donors of presomite stages all the material anterior to Hensen's node was implanted, from early somite stages either all anterior to the first somite, or in some cases all the tissue ventral to the gut cavity plus a varying amount of the forebrain. A series of grafts was also made from head-process stages in which the implants extended from the anterior opaque area to include a varying amount of the head-process. Because of the failure of hypophysis to differentiate in these grafts, with one exception, they are not described in detail in this paper.

DATA OBTAINED FROM GRAFTS

THE CAPACITY OF THE FOREBRAIN FOR DIFFERENTIATION

Grafts from implants of head-process stage. Series I.—The first problem to be investigated in regard to the forebrain was whether it would differentiate after isolation of prospective forebrain material during the head-process stage from all posterior and especially all notochordal material. Embryos of head-process and very early headfold stages were used for the first series of experiments. Figure 1 shows the types of implants. Type *a* consisted of most of the mate-

rial anterior to the anterior tip of the head-process up to the opaque area. Type *b* included the material behind *a* either back to the node level or including the node level. From eighty-five live hosts which received Type *a* implants and were alive at the time of opening, only twenty-nine grafts were obtained. Table I gives the list of structures differentiated in twenty-six of the Type *a* and twelve of the Type *b* grafts, together with measurements made before implantation. The

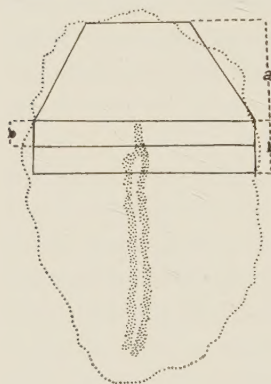


FIG. 1.—Outline showing implants for Series I.

twelve Type *b* grafts are from the same donors as twelve of the Type *a* grafts.

The three remaining grafts, of the twenty-nine mentioned above, were not listed in the table because the only tissue they contained consisted of a few vacuolated epithelial tubes and scattered cells. It was thought at first that these tubes were endodermal in origin, but later grafts of ectoderm or ecto-mesoderm alone were found to contain structures identical in appearance (see Table II*). Therefore it has been concluded that the vacuolated cells came either from material destined to

be skin epithelium or brain. An ectodermal origin seems more likely than a mesodermal one for various reasons. Pigment granules are present in similar tubes in two grafts of Series I (100-9, 87-9). In the latter they form part of the pigmented coat of the retina which, together with other forebrain tissue, is differentiated in this graft.

It will be seen from Table I that forebrain almost invariably differentiated from grafts of material anterior to the head-process or notochord, that is, from Type *a* implants, in head-process and early headfold stages. This was true even when material anterior to the apparent anterior end of the head-process was included in the Type *b* implant, as is known by measurements for some of the grafts. Even where measurements were not recorded of the distance of the cut from the anterior end of the head-process, care was taken not to cut too close to the visible end, since the head-process ends in fusion with the prechordal plate material. The latter was very seldom distinguishable, but from the almost invariable presence of mesodermal

structures such as cartilage and bone it seems necessary to conclude that part of it was included in the Type *a* implants.

The complete absence of notochord in the grafts from Type *a* implants suggests that none of the head-process material was included in such implants; but it is, of course, possible that an amount of material too small to differentiate or persist in a graft might be enough to induce the formation of nervous tissue if such induction is

TABLE II†

STRUCTURES DIFFERENTIATED IN GRAFTS FROM HEAD-PROCESS DONORS, SERIES I

NUMBER OF GRAFTS	TYPE	LENGTH OF HEAD-PROCESS IN UNITS	DISTANCE OF CUT FROM NODE IN UNITS	NUMBER OF GRAFTS IN WHICH STRUCTURES LISTED APPEAR														
				Brain Tissue	Prosencephalon	Mesencephalon	Eye	Epiphysis	Choroid Plexus	Ganglia	Epidermis	Notochord	Cartilage	Bone	Heart	Skeletal Muscle	Gut	Endodermal Glands
6	<i>a</i>	1-4	4-7	6	5	...	4	1	3	...	4	...	4	1	1	2	5	2(1T, 1L)
3	<i>b</i>			3	1	...	1	...	...	1	2	1	2	...	1	...	3	
7	<i>a</i>	5-14	6-14	6	5	...	5	...	2	...	4	...	5	2	2	1	7	5(5T, 2L)
2	<i>b</i>			2	1	1	1, 1?	1	2	2	2	1	2	...	1	1	2	1T
9	<i>a</i>	15-22	18-25	8	6	...	6	...	3	...	5	...	4	1	...	2	7	5(4T)
5	<i>b</i>			5	5	2	5	1	2	2	5	2	5	2	3	4	5	4(3L)
4	<i>a</i>	Early head-fold		4	1	...	1	...	1	...	2	...	3	1	...	...	3	2T
2	<i>b</i>			2	2	2	2	...	2	2	2	1	2	2	1	2	2	2(1T, 1L)

† KEY: *a*=grafts of implants anterior to head-process, *b*=of head-process level, T=thyroid, L=liver.

necessary. That notochord does not always differentiate even when head-process or node material was known to have been included in the implant can be seen from the data on Type *b* implants in Tables I, II, and III. The following arguments are thought more conclusive in establishing the non-inclusion of head-process tissue in the Type *a* implants. Wetzel (1929) has shown that the material anterior to Hensen's node in primitive-streak stages gives rise to the forebrain and that the anterior end of the head-process in later stages represents the original position of the node. The presence of eye in grafts from both *a* and *b* implants from the same donor, which

occurred five times in this series, would therefore indicate that head-process material was not included in the *a* implants; yet the brain tissue developed typically in such grafts. The case of a special graft (101-9) may also be mentioned here. For this graft measurement was made not only of the distance of the cut anterior to the head-process but also of the amount of medullary plate material included in the implant. This was found to be approximately 0.16 mm., or a little less. In the living blastoderm the area of the primordium of the medullary plate anterior to the node or early head-process measures between 0.32 and 0.47 mm. in anterior-posterior direction. This approximates Wetzel's measurements of the same area as far as could be told from scale drawings and reconstructions. It would seem quite conclusive, therefore, that no head-process material was included in the implant of the above-mentioned graft; yet again brain and eye differentiation were typical.

The histology of the grafts from head-process and headfold stages is summarized with that of primitive streak implants on page 213 and is figured on Plate I (Fig. 4).

Grafts from implants of primitive-streak stage. Anterior and node levels. Series II.—From the data of grafts of the head-process stage it seemed possible that the presence of prechordal mesoderm might be sufficient to induce brain formation if induction is necessary. For this reason a series of grafts of definitive primitive-streak stages was made. As shown by Wetzel (1929) and Adelmann (1922), prechordal mesoderm is present anterior to Hensen's node in these stages. The *a* implants of Series II were similar to those made by Hunt (1931a), including the area between the node and the anterior limit of the pellucid area (see Fig. 2). Three grafts (94-15, 95-3, and 105-13) were from implants of ectoderm or mesectoderm only. Two of these are indicated (by an asterisk) in Table II; the third contained only epithelium and fibrous tissue. The only difference in technique from Series I was that measurements were taken of the length of the primitive-streak and of the distance of the cut from node and pit. Type *b* implant for this series included the node level only. See Figure 2 for the types of grafts. From forty-seven live hosts twenty grafts were obtained of which twelve, and their reciprocals where recovered from *b* implants of the same donors, are summarized

in Table II as to the structures which differentiated. The other eight grafts were like the six of Series I mentioned above, containing only vacuolated epithelial tubes. Of these eight, two were from implants of ectoderm or ectomesoderm only, the endoderm having been removed very easily as a continuous layer before grafting. As noted by Hunt (1931a), the endoderm separates very readily from the ectoderm; and in some cases complete separation occurs without the efforts of the operator. This separation can always be noted along the posterior cut edge of the *a* implant; and, as in Hunt's operations, a bit of the opaque area was sometimes included in such implants in order to insure the adherence of the germ layers to each other.

The amount of material differentiating in grafts of Type *a* implants of this series was slight. As can be seen from the table, epithelium was the most constantly recurring structure. Practically all of the grafts included in the table showed tubules of vacuolated epithelial cells like those of the eight grafts mentioned above. Their probable origin has been suggested under Series I. Brain tissue could be positively identified in only three cases (see Fig. 5), in one of which it was represented solely by a small mass of the pigmented coat of the retina. Its differentiation corresponded to that of brain in grafts of head-process stages. Masses of unidentifiable tissue which it was thought might be nervous tissue (indicated in Table II by a question mark) were present in several cases (see Fig. 6). The similarity of this tissue to that found at the edges of brain vesicles in grafts from older donors suggested this interpretation. It may be noted here that the three cases of actual differentiation of brain tissue, and two of the three of tissue resembling nervous tissue, appeared in grafts from donors with primitive-streak lengths in the range of 2.16–2.76 mm., 60–80 units. The length of the primitive-streak at the time of the origin of the head-process differs in different individual blastoderms but approaches the range of 2.00–2.76 mm. This statement is made on the basis of measurements of forty embryos in which the

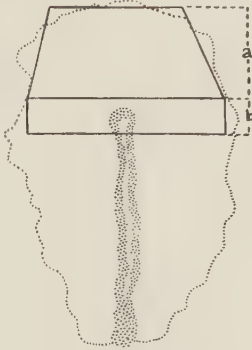


FIG. 2.—Outline showing implants for Series II.

head-processes had just begun, being 0.16 mm., or less, in length. Three of the forty had primitive-streaks only 1.83 mm. long, eighteen were between 2.00 and 2.33 mm., sixteen between 2.33 and 2.50 mm., one 2.76 mm., and two 3.00 mm.

Grafts from implants of primitive-streak stages. Antero-lateral and node-streak isolations. Series III.—A third series of experiments, made like the second on primitive-streak stages, gave comparable

TABLE II†

STRUCTURES DIFFERENTIATED IN GRAFTS FROM PRIMITIVE-STREAK DONORS. SERIES II

NUMBER OF GRAFTS	TYPE	LENGTH OF PRIMITIVE-STREAK IN UNITS	DISTANCE OF CUT		NUMBER OF GRAFTS IN WHICH STRUCTURES LISTED APPEAR																
			From Node	From Pit	Brain Tissue	Prosencephalon	Mesencephalon	Eye	Epiphysis	Choroid Plexus	Ganglia	Epithelium	Fibrous Tissue	Notochord	Cartilage	Bone	Heart	Skeletal Muscle	Gut	Endodermal Glands	
6	a	40-55	1-6	3½-8½	*1?	...	...	...	...	...	...	6	4	...	...	...	...	2?	...		
1	b				1	...	...	...	...	...	1	1	...	...	...	1	1	1	1 (1T, 1L)		
6	a	60-80	2½-6	4½-8	3†+2?	*1	...	*1	...	...	3	1	...	...	...	...	...	4	...		
3	b				3	2	1	2	1	2	2	3	...	2	2	...	1	2	2		

† KEY: *a* = grafts of implants anterior to node, *b* = of node level, T = thyroid, L = liver.

* Implant for graft contained no endoderm.

results. In this series the Type *a* graft contained a larger area of prospective medullary plate material than the Type *a* graft of Series II. Node and streak material was excluded, but most of the material up to the opaque area and all lateral to the streak for the anterior half of its length was included. The *b* implant included the node, a bit of the tissue just anterior to it, and approximately the anterior half of the primitive-streak. Figure 3 illustrates the type of cut made, while Table III lists the structures which differentiated from both Type *a* and Type *b* implants and gives the measurements made at the time of operation.

It is again noticeable that, with one exception, the Type *a* grafts in which nervous tissue appeared were from donors with fairly long

primitive-streaks, between 2.16 and 2.83 mm. Apparently a certain stage was usually reached in all grafts of implants from primitive-streak donors before differentiation of brain tissue would occur in the absence of node and streak material. The one exception to this is the case of the graft 99-11, which contained a small amount of brain tissue, and for which the implant came from a blastoderm with a primitive-streak approximately 1.6 mm. in length. It had been noted at the time of operation that the cut had been made very close to the edge of the streak some distance below the region of the node. In the case of one Type *a* graft (78-3) a small bit of tissue resembling notochord appeared. Since the donor for this graft had a primitive-streak of 2.48-2.64 mm., it may be that the head-process had begun to form and that the tip of it was included in the implant.

It was not possible to determine whether the brain present in these grafts was fore-, mid-, or hindbrain except when eye or epiphysis or the stratification typical of midbrain appeared. The percentage of takes from Type *a* implants of this series was better than of Type *a* implants in Series I and II, where only the material anterior to the head-process or to Hensen's node was included (see Table IV). This was thought due to the larger amount of material included in Type *a* implants of the present series, a condition which perhaps allows vascular connections to be more readily established at the time of implantation.

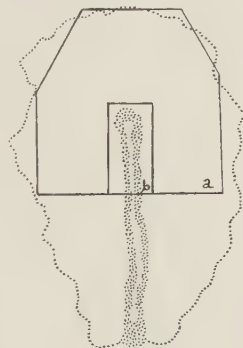


FIG. 3.—Outline showing implants for Series III.

THE HISTOLOGY OF GRAFTS OF SERIES I, II, AND III

The histology of the tissues in these grafts is essentially the same as that described by Willier and Rawles (1931) from implants of total blastoderms of the same ages. The chief differences between grafts from implants of anterior or anterior and lateral material (Type *a*) and those including node level or node and streak (Type *b*) are that a smaller number of tissues and a lesser amount usually differentiates in the former and that the arrangement thereof is

PLATE I

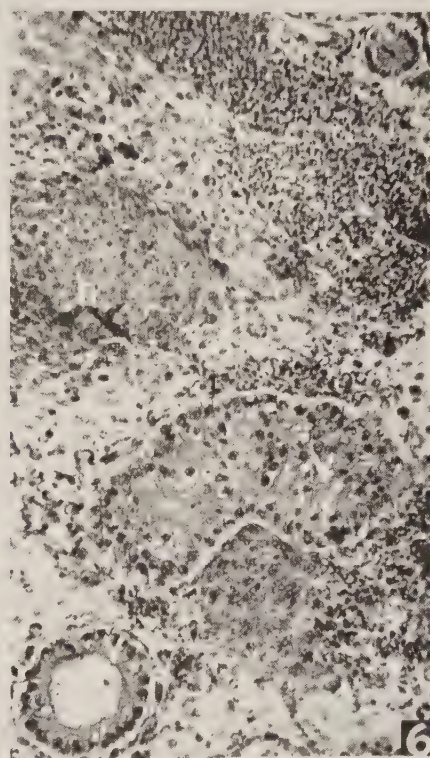
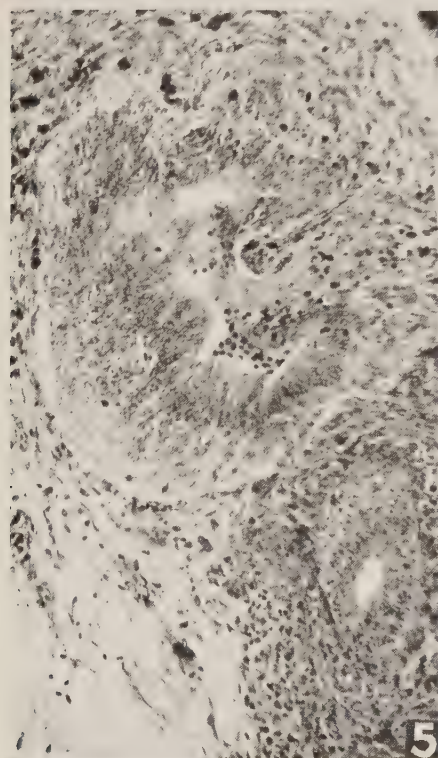
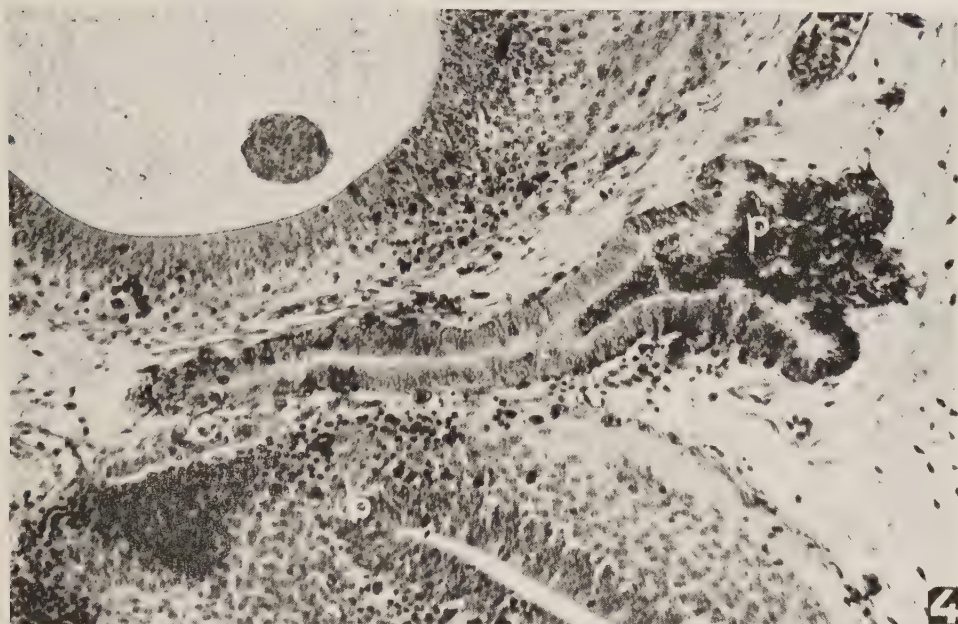
b=brain, *e*=epidermis, *h*=hypophysis, *n*=nervous tissue, *p*=pigmented layer of retina, *v*=vacuolated cells.

FIG. 4.—Photomicrograph of section of graft 101-9. Donor with head-process of approximately 0.03-0.06 mm. Implant from level anterior to head-process (Series I, Type *a*). Age of grafted tissue, 8 days. $\times 210$.

FIG. 5.—Photomicrograph of section of graft 92-7. Donor of primitive-streak stage. Implant from level anterior to primitive-streak (Series II, Type *a*). Age of grafted tissue, $8\frac{1}{2}$ days. $\times 210$.

FIG. 6.—Photomicrograph of section of graft 95-3. Donor of primitive-streak stage. Implant of ecto-mesoderm anterior to primitive-streak (Series II, Type *a**). Age of grafted tissue, $8\frac{1}{2}$ days. $\times 210$.

PLATE I



usually less normal, rather than that the degree of differentiation attained is lower. This is true also for a comparison of the grafts of

TABLE III†

STRUCTURES DIFFERENTIATED IN GRAFTS FROM PRIMITIVE-STREAK DONORS. SERIES III

NUMBER OF GRAFTS	TYPE	LENGTH OF PRIMITIVE-STREAK IN UNITS	TYPE <i>b</i> IMPLANT		DISTANCE OF CUT ANTERIOR TO NODE IN UNITS	NUMBER OF GRAFTS IN WHICH STRUCTURES LISTED APPEAR																
			Length	Width		Brain Tissue	Prosencephalon	Mesencephalon	Eye	Epiphysis	Choroid Plexus	Ganglia	Epithelium	Notochord	Cartilage	Bone	Heart	Skeletal Muscle	Gut	Endodermal Glands		
7	<i>a</i>	40-55			2-4	*1	..	..	..	..	..	..	7	..	..	..	3	1	7			
4	<i>b</i>		20-35	10-16		2	..	1	..	..	..	..	4	1	..	..	2	1	3	1L		
13	<i>a</i>	60-85			2-5	7+2?	3	..	3	2	3	..	12	1	3	..	7	1?	12	4(2T, 3L)		
4	<i>b</i>		30-40	13-16		2	..	1	..	..	..	..	2	..	3	..	2	..	4	1T		

† KEY: *a* = grafts of implants anterior and lateral to node and streak, *b* = of anterior half of streak including node, T = thyroid, L = liver.

* Implant cut close to streak.

TABLE IV

COMPARATIVE DATA FOR TYPE *a* IMPLANTS OF SERIES I-III

Stage of Donor	Type of Grafts	Number of Hosts Alive	Total Number of Grafts Obtained	Number Containing Nervous Tissue or Tissue Similar Thereto	Percentage of Grafts Obtained	Percentage Containing Nervous Tissue or Tissue Similar Thereto
Head-process...	Anterior to head-process, Series I, Type <i>a</i>	85	29	24	34.1	82.8
Primitive-streak.	Anterior to node, Series II, Type <i>a</i>	47	20	6	44.7	30.0
Primitive-streak.	Anterior and lateral to node, Series III, Type <i>a</i>	28	20	10	71.4	50.0

primitive-streak donors as compared with those of head-process donors.

PLATE II

b=brain, *e*=epidermis, *h*=hypophysis, *n*=nervous tissue, *p*=pigmented layer of retina, *v*=vacuolated cells.

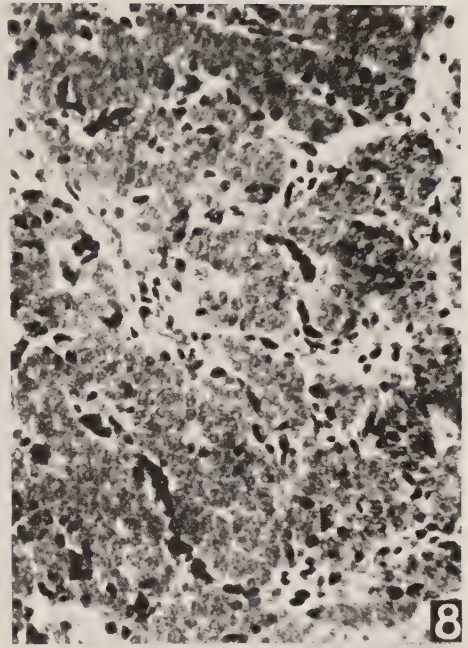
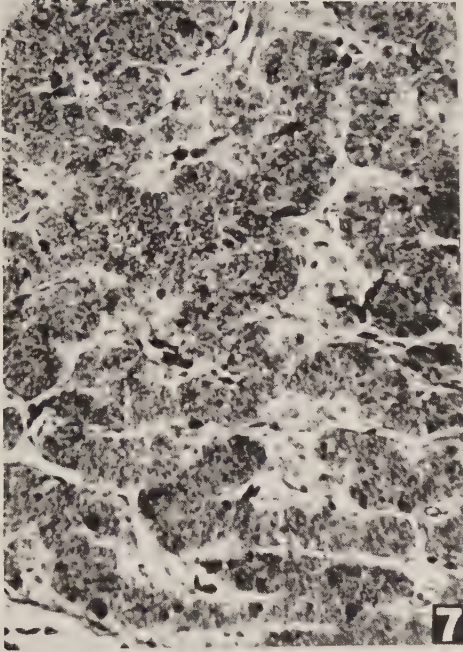
FIG. 7.—Photomicrograph of hypophysial cords of normal chick embryo of 12 days' incubation. $\times 275$.

FIG. 8.—Photomicrograph of section of graft 5-20 showing hypophysial cords. Age of donor, 3 days; of grafted tissue, 13 days. $\times 275$.

FIG. 9.—Photomicrograph of section of graft 19-7. Age of donor, 12 somites; of grafted tissue, $9\frac{1}{2}$ days. $\times 275$.

FIG. 10.—Photomicrograph of section of graft 82-10. Age of donor, head-process stage; of grafted tissue, 10 days. $\times 275$.

PLATE II



Figures 4, 5, and 6 give some idea of the amount of differentiation attained in Type *a* implants. The brain occurs typically in the form of vesicles with a definite lumen bounded by columnar-shaped cells. The internal limiting membrane is in almost all cases distinct; and between it and the first nuclei there is a narrow non-nucleated margin, the internal ependymal layer, the width of which is approximately uniform around the lumen. Mitoses are usually fairly numerous in the nuclei lying closest to the lumen. The cells lining the lumen give an appearance of pseudostratification; their cytoplasm is comparatively dense, being bluish gray in color with the stain used. External to the area just described is a looser-looking area which varies greatly in width in different grafts and in different regions within the same graft. Variation in width of this layer is also found in the normal chick. Here the nuclei are fewer in number and are scattered, seemingly connected with fiber-like strands of cytoplasm. The general appearance of this area is spongy or veil-like. In some cases more definite fibers appear to be present. It is in this area that signs of disintegration are most often found in the grafted tissue. It may be heavily infiltrated with blood cells or may look as if eaten away, large holes being present with only a few strands of tissue and a few isolated nuclei separating them. Outside this area there may or may not be a definite external limiting membrane. Sometimes the tissue appears to be unconfined and to lose itself among the strands of chorio-allantoic tissue or that of other structures in the grafts. Compared with the forebrain of a normal chick of 9 days' incubation, the latter is found to have the same layers; but the cells seem smaller and much more closely packed. In the veil-like layer in the normal brain the fibers are perhaps more distinct—the structure not quite so spongy; this may also be simply a matter of fewer cells in the brain tissue of the graft. Comparison with the normal in the case of the brain is not especially helpful in determining the degree of delay in differentiation caused by the implantation, since the histological structure of the forebrain of chicks of 7–9 days is essentially similar.

As far as eye is concerned, the pigmented layer of the retina is sometimes the only part appearing in grafts from implants excluding all node and streak (Type *a* in all three series). It is, however, present

in all cases where eye is recorded. The pigmented layer in some cases is a single row of cells which vary in shape from cuboidal to low columnar. Sometimes it is very much twisted, owing probably to abnormal growth factors in the graft; and in these cases the mass of it may appear to bud off small tubes which may, in some grafts, be cut in cross-section. In all cases the cytoplasm is literally packed with dark-brown or black pigment granules, so that cell outlines and often nuclear outlines cannot be distinguished. The difference from the normal of the same age is that in the graft retina the cells seem larger and less numerous, and their arrangement in foldings and masses of tissue is abnormal.

The sensory layer of the eye, when present, corresponded to that in the normal embryo of 8 days of incubation. The grafted tissue was between $9\frac{1}{2}$ and 10 days of age. Thus there was a delay of approximately 2 days in the eye of the graft, and this delay was measured by the absence of the outer molecular layer. The inner and outer nuclear layers were thus fused except that in places a small light line, evidence of the beginning separation, was present. This condition is also present in areas of the sensory layer of the retina of the 8-day chick.

The ectodermal epithelium found in the grafts was sometimes stratified and sometimes resembled oral and nasal epithelium. No attempt was made to identify the various types in the individual grafts, since it did not seem necessary for the purposes of this investigation. The vacuolated type of epithelial tubes has already been mentioned. Some grafts of all three series showed stratified epidermis and feather germs, the latter being in general better developed in grafts of implants of head-process stages than in the corresponding implants from primitive-streak stages.

The endodermal glands were identified by their connection with gut tissue. The latter consisted of tubes of cells with characteristically darkly staining nuclei sometimes surrounded by a band of muscular or fibrous tissue. Individual identification of pharynx and various gut levels was not made, although they were differentiated in some of the grafts. No evidence of trachea, with its characteristic arrangement of cartilage, was noted. Thyroid and liver were distinguished by a series of criteria; the level of gut with which they

were connected, the presence of colloid which is sometimes found in the thyroid, and the darkly staining nuclei and vacuolated cells characteristic of the liver, as well as by the differing arrangement of the cords of cells which are more solid and more infiltrated with blood in the case of liver than of thyroid.

Notochord did not occur with great frequency, but was found in contact with nervous tissue when it did occur. It had the typically vacuolated structure characteristic of its appearance in the normal embryo. Cartilage and bone were found in most of the grafts with the exception of those in Series II from Type *a* implants. Heart, where present, was usually a mass of cardiac tissue which possessed a lumen in a few cases only.

THE DIFFERENTIATION OF THE HYPOPHYSIS

Previous work (Stein, 1929) has shown that the hypophysis will differentiate in grafts from embryos ranging in age from 5 days to the headfold stage. Table V is a summary of grafts made for the previous study and of additional grafts of implants from headfold and head-process stages recently made.

The amount of tissue included in the implants varied according to the age of the donor. For the older embryos this was reported in the 1929 paper. For the younger, it has been given earlier in this paper.

The percentage differentiation of the hypophysis decreases steadily with the age of the donor, as can be seen by consulting Table V. A most noticeable drop, from 80 per cent to 55 per cent, comes between the fifteen- to twenty-two-somite and the eight- to twelve-somite stages. This coincides very nicely with the first appearance of the hypophysis as a thickening of the ectoderm, which, according to Adelmann (1922), occurs in the fifteen-somite embryo.

The structure of the hypophysial cords in grafts from all stages of donors is essentially the same. They are two cell layers in width in their thinner portions, and three to four where they branch or curve on themselves. Measurements with an ocular micrometer show that in the grafts of all stages and in normal 11- and 12-day embryos these cords are actually the same width. This is also evident for the grafts from the Figures 8, 9, and 10. No cytological

differences are evident, but these would not be expected in tissue of this age. The great difference between hypophysis in grafts from younger and those from older donors is in the amount of tissue present. As would be expected, this is less in grafts from younger donors. What makes the difference even more striking is that where there is a small amount of hypophysial tissue the appearance of the gland as a complexly branched structure is altered to that of a few cords

TABLE V
DATA ON THE CAPACITY OF THE HYPOPHYSIS FOR DIFFERENTIATION

AGE OF DONOR†	NUMBER OF GRAFTS IN WHICH STRUCTURES LISTED APPEAR																					
	NUMBER OF GIFTS		HYPOPHYSIS	INFUNDIBULUM	Brain	Epiphysis	Eye	Lens	Choroid Plexus	Ganglia	Epidermis	Epithelial Ingrowths	Feather Germs	Skeletal Muscle	Heart	Cartilage	Bone	Notochord	Gut	Thyroid	Liver	Percentage of Appearance of Hypophysis
3-5 days...	16	15	15	16	2	4	..	7	10	16	10	15	14	..	16	16	4	...	...	...	...	94
238-54 hr...	9	7	6, 3?	9	..	7	1	1	..	7, 1?	6	3	7	..	9	6	...	1, 2?	...	...	...	78
158-228....	10	8	7, 1?	10	..	7	3	..	2	8	7	5	7	..	9	8	...	1	1	..	...	80
88-128.....	9	4, 1?	2, 4?	9	1	9	6	2	..	7	4	4	4	1	8	5	...	1	1	..	...	55
18-78.....	15	3	1, 3?	15	3	12	3	6	1	15	6	10	5	3	12	5	1	9	4	1	...	20
Headfold and head- Process...	20	2, 1?	2	20	..	11	1	12	5	20	2	8	9	10	17	9	5, 2?	19	10	9	15	

† Somites are indicated by "s."

with budlike projections, or to an aggregation of small lumplike masses of tissue, depending on the section. This results in there being fewer spaces for mesenchyme and blood capillaries and so further alters the picture characteristic of the gland in the normal embryo.

Hypophyses from donors up to fifteen somites are in all cases connected with oral epithelium, and some of them still show a large central cavity from which the cords bud. This is thought to be the remains of the lumen of Rathke's pocket. The connection with oral epithelium was not present in a 9-day-old normal embryo, but its presence in the 10-day and older grafted tissue may be the result

of mechanical conditions preventing normal growth relations in the grafts rather than an evidence of retardation. This may also be true for the remains of the lumen of Rathke's pocket, or the presence of the latter may be evidence of a real retardation. It is present in the 7-day-old normal embryo but has disappeared by 9 days, while the grafts in which it was present were 9-10 days in age of grafted tissue.

The grafts in which the hypophysis has not differentiated seem to have no other common factor with which the absence of the hypophysis could be correlated. Four grafts of the somite stages show no differentiation of epidermal structures; but the remaining thirty-one grafts which lack hypophysis have well-differentiated epidermis, and eight of these, scattered through a range from head-process to 4-day donors, have cordlike outgrowths (not hypophysial) from epithelium similar to oral epithelium and connecting with stratified epidermis. Fifteen have easily recognizable feather germs; five have a lens associated with eye tissue. Consequently, in most of the cases skin ectoderm was differentiating, as far as other structures than hypophysis were concerned. All the grafts have brain tissue; and, since most of them have pigmented cells of the retina, this was interpreted as diencephalon. The actual identification of an infundibular process is felt to be unreliable in the absence of hypophysis. There were in some cases brain outgrowths which resembled those occurring in the region of the hypophysis when the latter was present in grafts, but they could not be specifically recognized as infundibular. On the other hand, hypophysis, while always found in close relation to brain tissue, was not always associated with a typically normal infundibular process.

The amount of tissue included in the implant did not seem to have any connection with the presence or absence of hypophysis in the grafts. For all of the head-process and headfold stages summarized in Table IV the implant included all the tissue anterior to Hensen's node. However, another graft, 47-8, for which the implant extended posteriorly to include only the anterior half of a head-process about 0.6 mm. long, contained well-differentiated hypophysis. The implants for the three grafts containing hypophysis from one to seven somite donors included only the tissue ventral to the gut

cavity and the ventral part of the forebrain. The other grafts from donors of the same stage, though from implants containing the same amount of tissue in four cases and including also the dorsal forebrain in six, plus the midbrain in two cases, had no hypophysis present in them. The grafts showing non-differentiation from older donors were in all cases from implants identical in amount of tissue with those which furnished hypophysis in other grafts.

DISCUSSION

THE LOCATION OF FOREBRAIN MATERIAL

Wetzel (1929), in a series of vitally stained embryos, found that the material giving rise to the forebrain lies anterior to Hensen's node in the primitive-streak stage, and the anterior-posterior length of the area would seem from his measurements to be not more than 0.4-0.5 mm. In his vitally stained embryos, if the stain was placed anterior to the node in the definitive primitive-streak stage, the color appeared in the forebrain; if the node level alone was colored, the forebrain remained unstained but the notochord and more or less of the neural tube posterior to the forebrain was stained. The former type of experiment used by Wetzel, that of placing the stain anterior to the node, was repeated by the present writer on a small number of embryos. The technique of Kopsch, confining the agar in very fine glass tubes which could be held against the blastoderm and a fairly distinctly localized mark obtained, was used. The results agree with those of Wetzel, since forebrain was found to be stained in the four cases in which development had been normal and the stain was deep enough to determine its location at the time of examination.

The morphological studies of Kingsbury and Adelman (1924, pp. 247-48) have brought them to the conclusion that among vertebrates the prechordal medullary tube "furnishes the 'first primary brain vesicle' from which the optic vesicles arise by a continuation of the bilateral expansion." Kingsbury (1920, 1922, 1931) considers that the epichordal brain, as opposed to prechordal, extends from the medial floor of the midbrain backward. The chick embryo was one of the types of vertebrate embryos used by him in this study.

In this paper it has been shown that forebrain will differentiate in

chorio-allantoic grafts in almost every case from implants of the material anterior to the head-process, and in several cases from implants of the material anterior to the node in the stage just before or about the time the head-process has begun to form. These results are thus in line with the idea that the forebrain material, including eye material, is located anterior to the head-process in the normal chick embryo of that stage, and anterior to Hensen's node in the definitive primitive-streak stage. The experiments are not suitable for defining the posterior limit of the forebrain material, since in the attempt to insure exclusion of the tip of head-process or node from the implants the cut for isolation was usually made somewhat anterior to the apparent anterior limit of these structures.

This presence of some anterior material in the implant may explain the presence of forebrain as denoted by eye or epiphysis in many of the grafts from the posterior level which included also either all of the head-process anterior to the node, or the node level, or both, depending on the stage used (level *b* in all grafts). This explanation would also seem adequate for the presence of forebrain structures in Hunt's grafts of the node level of definitive primitive-streak blastoderms. It does not account for the presence of hypophysis reported by Hunt in the same grafts, if, as seems likely, the hypophysis material is located anterior to that giving rise to forebrain. The absence of hypophysis from eleven grafts of implants of the same level made by the writer and its rare occurrence in grafts of all the material anterior to the node from later stages up to nine somites suggests that the structure identified as hypophysis by Hunt may be something else. Thyroid sometimes has a similar cordlike appearance in grafts, and Hunt makes no record of the appearance of the latter structure. In a previous paper (Stein, 1929) the author reported hypophysis as present in a graft of a presomite stage. This has since been found to be thyroid. Hunt has also stated (personal communication) that the hypophysis recorded in his grafts was not always in close relation with the brain. From entire blastoderms Willier and Rawles (1931) obtained only three cases of hypophysis in twenty-eight grafts of primitive-streak and head-process stages, while Hunt reports seven cases out of twenty-five in grafts of the node level alone of the definitive primitive-streak stage. Even if

hypophysis is present in some of these cases, the possibility of its being the result of induction by the forebrain material is not disproved.

Therefore, in any case, Hunt's material does not exclude the possibility of forebrain material being definitely located in front of Hensen's node in the primitive-streak stage. It may, of course, be possible that the removal of the anterior level stimulates the level behind it, the node level, to form anterior structures for which the material was lacking in the implant. This would mean that the node level is more or less totipotent. However, Hunt's later experiments (1931*b*) in transecting the node and implanting parts of it to the chorio-allantoic membrane suggest that the node, rather than being totipotent, is in itself a mosaic, as indicated by the fact that the anterior half of the node level gave anterior structures only, the posterior half posterior ones. The first explanation for the presence of forebrain structures in both my grafts and those of Hunt from implants of the node level—viz., that a part of the material anterior to the node and normally giving forebrain was included in such implants—would therefore seem the more likely one.

It may be mentioned here that the presence of a structure in a graft does not mean that the structure is necessarily present in its entirety, that is, for example, that a complete forebrain or eye is present in the foregoing grafts. Histologically, of course, it is possible to say that forebrain tissue, pigmented and sensory coat of the eye, etc., are present; but there is no reliable criterion at the age attained by the grafts for judging whether or not part or all of the telencephalon is present. Shape is too abnormal in most cases; and the amount of material is not as great as that attained in the normal embryo. The latter is true even for grafts from implants which included the entire primordium of a structure from a later donor, as, for example, in the experiments of Hoadley (1924) on the eye, nasal region, otic region, and mesencephalon. Hoadley (1929) has pointed out that differentiation of structures in chorio-allantoic grafts takes place regardless of the retardation of cell multiplication.

Taking into account all the facts discussed above, there seems to be definite positive evidence for the localization of forebrain material anterior to Hensen's node in the definitive primitive-streak stage

of the chick embryo. Also, as far as I have been able to discover, there is no positive evidence against this conception.

THE DETERMINATION OF FOREBRAIN MATERIAL

It has been suggested by Hunt (1931*a*, p. 423), in his study of the rôle of Hensen's node in the development of the chick embryo, that "the head-process induces the medullary plate to form in the chick." From a study of grafts from implants of the entire pellucid area of blastoderms ranging from the definitive primitive-streak stage to the eleven-somite stage, Willier and Rawles (1931, p. 460) state that it "is somewhat well brought out" in such grafts "that the notochord may be of importance in bringing about the development of the medullary plate." They note that, "as a rule when any part of the central nervous system, except the forebrain, differentiates, it is in more or less intimate association with notochord." This is, of course, the normal situation, since the notochord in the chick embryo extends approximately up to the level of the infundibulum, separated from the latter, according to Kingsbury (1922), by the extent of the prechordal plate material. As has previously been discussed, the localization of forebrain material in the definitive primitive-streak stage seems to be anterior to Hensen's node; and this corresponds with its position anterior to the notochord in later stages, since, as shown by Wetzel (1929), the position of Hensen's node in the primitive-streak stage is that of the anterior end of the notochord in later stages. The present experiments of implanting to the chorio-allantoic membrane material anterior to the head-process stages have shown that the forebrain material will differentiate after isolation from all notochord or head-process material. This is true even for implants of the anterior material from embryos in the earliest recognizable stage of the head-process. Therefore, it would seem that development of the forebrain is not dependent on the presence of notochord or head-process after the latter has become visible.

The differentiation of forebrain from the level anterior to the node in the definitive primitive-streak stage occurred normally in only three grafts (Series II); and in only one of these, in which the pigmented layer of the eye formed, could this be identified histologically as forebrain. The others, however, almost undoubtedly represented

forebrain material, since no level behind forebrain could reasonably be expected. Hunt (1931a) found nervous tissue in only one of fifteen grafts of this same type, and he concluded that node tissue was probably present in the implant. This possibility is perhaps not entirely ruled out for the cases of the present study if the anterior extent of the node is considered as indefinite in morphological indication. Nevertheless, the presence of brain in these grafts from material anterior to the visible node, as well as its presence in six grafts which included lateral as well as anterior medullary plate material, suggests that the material destined to give rise to forebrain may be determined to the extent that it will self-differentiate into typical nervous tissue even in the definitive primitive-streak stage. The fact that inclusion of some node cells in grafts from the anterior level resulted, according to Hunt, in differentiation of forebrain in all cases may be due to the inclusion of more prospective forebrain material or of part of a center of rapid proliferation rather than to any influence of future notochordal material included. Since notochord never differentiated in the grafts, it is possible, although not proved, that no prospective notochord material was present in the implants.

Wetzel (1929) was able to measure the limits of the "Scheibe," future medullary plate material, as an area of thickened ectoderm, by the histological study of sections of total blastoderms. He did this for the definitive primitive-streak stage and also for earlier stages having a somewhat elongated, as opposed to a broad, primitive-streak, but with the pellucid area round rather than pear-shaped. The medullary plate material therefore becomes visibly determined before the appearance of the head-process. Since actual determination of a structure, as proved by self-differentiation, usually occurs before morphological differentiation, this would indicate that medullary plate, in part at least, may be already determined in the somewhat elongated primitive-streak stage.

Various homologies can be found between the results obtained here and those gathered concerning the determination of medullary plate in Amphibia by various German investigators. Bautzmann (1926) and others have demonstrated the power of the dorsal lip of the blastopore, future chorda-mesoderm, for the induction of the

medullary plate in various species of Anura and Urodeles. Rauber (1876) first spoke of the primitive-streak of the chick as a slitlike blastopore. Since that time the homology of Hensen's node in the chick to the dorsal lip of the blastopore of Amphibia has been stressed by Adelmann (1922), Wetzel (1929), and others. In the Amphibia it is the chordamesoderm of the archenteron roof which is responsible for the medullary plate induction, rather than the endoderm. As pointed out by Gräper (1929), the process of gastrulation in the chick differs from that of Amphibia in being divided into two periods. A fertile unincubated chick egg is usually two-layered; it has both ectoderm and endoderm; gastrulation has already begun. The material which will give the future mesoderm of the embryo, however, is still on the surface and only later, with the narrowing of the broad primitive streak, comes to lie beneath the ectoderm of the future medullary plate. This it does, as shown by Wetzel (1929) by the vital staining method and by Gräper (1929) in moving-pictures of the vitally-stained blastoderm, by streaming medianly, turning under in the primitive groove region and spreading out under the ectoderm from the primitive folds. At the anterior end of the primitive-streak, at the future Hensen's node, prechordal mesoderm appears to form as a direct outgrowth from the thick knot where ectoderm and endoderm are fused. The time of origin of this mesoderm, according to Wetzel (1929), is when the broad primitive-streak is transforming into the narrow, somewhat elongated, streak. It is possible that determination of the forebrain material in the chick may occur or begin at the time of origin of the prechordal mesoderm.

There are evidences in the present study, however, which support the idea that determination of the forebrain material occurs later in development than the time of origin of the mesoderm. In grafts of the level anterior to the node in the definitive primitive-streak stage, as compared with those anterior to the head-process in the latter stage, there is a very decided decrease in the percentage which contain brain tissue. The difference is striking even if all possible cases of nervous tissue differentiation from the former stage are considered.

Also, as has been pointed out in the descriptive part of this paper, VOL. VI, No. 2, APRIL, 1933]

the presence of easily recognizable nervous tissue from implants of the definitive primitive-streak stage is almost entirely confined to those of a range of length of primitive-streak corresponding to that during which the head-process begins. These facts would seem to be an indication of the definite time at which the medullary plate—at least the future forebrain material—becomes determined. However, the mesoderm is already present underneath the ectoderm at this stage, and the analogies mentioned above make it not unlikely that the initial period of determination would be that of the spreading-out of the mesoderm. Also, there were a few exceptions in the author's grafts in which nervous tissue differentiated from donors with primitive-streaks shorter than the range of length at which the head-process begins. Rudnick (1932), in this laboratory, has grafts containing brain from implants lateral to node and streak in similar early stages. Forebrain has been identified in two of these by the presence of eye or epiphysis. However, all but three of Rudnick's implants from early stages were cut closer to the node and streak than mine. Also, the one graft of mine showing definitely recognizable brain material came from an implant cut very close to the streak.

The conception of a gradual process of determination culminating in the stage of the formation of the head-process would seem to fit all the data of the present study. It is conceivable that the greatly increased frequency of forebrain differentiation in grafts of implants anterior to the head-process over that in those of implants anterior to the node in the primitive-streak stage may be due to an increase in the degree of determination correlated with the appearance of the head-process. Some experiments of Goerttler (1927) led him to the opinion that there was a certain degree of determination in the medullary plate of certain species of *Amphibia* even before gastrulation was complete. This seems to be brought out also by the experiments of Holtfreter (1929), who found that presumptive medullary plate of blastulae and early gastrulae of various *Amphibia* transplanted into the lymph sacs or body cavity of an adult would differentiate into vesicles with neural-tubelike structure and possessing neurofibrils.

The absence of brain in most of the implants from early donors might be explained on the assumption that determination is taking

place during this entire period but that the operation of isolating a section and transferring it to the chorio-allantoic membrane may prevent its indication in many of the grafts from earlier donors of this stage. Gräper (1926) has noted that the effect of even slight exposure to harmful agencies, such a change of temperature, light, etc., may cause a short regression in development, after which the egg may recover and again proceed in a forward direction. If this is true, we should expect a much more drastic effect from removing the blastoderm from the yolk, cutting it, and placing it on the chorio-allantoic membrane, where, as shown by Henderson (1930), it does not become incorporated for some time. There is also the possibility that the amount of prospective medullary material included was in some cases too small to differentiate. This suggestion receives some support perhaps from the fact that brain was more likely to develop in implants in which material lateral, as well as anterior, to the node was included.

It is, of course, true that in all the grafts of the primitive-streak stage mesoderm was probably included, even though it did not always differentiate. This allows for the possibility of induction occurring within the graft if we assume that the mesoderm causes induction. However, it seems unlikely that induction would begin to occur later than the spreading of mesoderm. Whether or not determination of the medullary plate material has begun earlier could perhaps be discovered by grafts from implants of prospective medullary plate ectoderm anterior and lateral to the streak in the stage when the latter is a broad tongue of cells and the blastoderm is still two-layered. Grafts of various levels of prenodal stages have been made by Hoadley (1926). While these prove self-differentiation of the level implanted, they do not prove it of single structures in the grafts. Forebrain differentiation, for example, in his grafts as well as in mine, may depend on the presence of mesoderm, since by including node or streak level he included the source of such mesoderm.

It should be noted here that the importance of the node in the process of development is not questioned. The node is the center for the proliferation of the prechordal and notochordal mesoderm; and even if determination should be present before this mesoderm is proliferated, such determination may still depend upon influences

radiating from the node or from prospective node tissue at an earlier stage.

The experiments reported in this paper, for the chick, are not considered equivalent to those done in *Amphibia* in transplanting presumptive medullary plate into the region of presumptive epidermis. For this reason it is not considered that determination, in the sense of irreversible determination, has been demonstrated by them. However, it can be stated from these and other experiments that there is evidence of a mosaic pattern in at least part of the medullary plate material in the stage of the definitive primitive-streak. This, according to the idea expressed by Gilchrist (1929, p. 551), "indicates that determination has begun" while "capacity for regulation reveals it is still in progress." Chorio-allantoic grafting does not seem to the writer a method capable of revealing capacity for regulation.

CAPACITY OF THE HYPOPHYSIS FOR DIFFERENTIATION

The capacity of the hypophysis for differentiation in chorio-allantoic grafts has been found to be very slight from implants of donors younger than eight to nine somites, and fairly constant only from donors over fifteen somites, the time at which morphological indication appears. It is true that Hunt has reported hypophysis as differentiating in seven grafts out of twenty-five of the node level of the definitive primitive-streak stage. The possibility that this may not be hypophysis in all cases seems fairly strong and has been discussed in a previous section.

The absence of hypophysis, as previously mentioned, does not seem to be correlated with the presence or absence of any other structures in the graft with the exception of brain. Hypophysis has never been found in grafts lacking brain; but, on the other hand, the presence of normal brain tissue, even in grafts from implants containing the floor of the diencephalon (or the tissue which will give rise to it), does not insure the presence of differentiated hypophysis. Yet, it is the floor of the diencephalon which gives rise to the infundibulum, and the latter is the part of the brain normally associated with the hypophysis. It is possible, as suggested previously (Stein, 1929), that the hypophysis is peculiarly dependent for its differentiation on the normal arrangement of structures and mate-

rials and that this is prevented in some way in the grafts. Kingsbury and Adelmann (1925, p. 277) state that "one of the important factors in the development of the hypophysis is the formation of a mesoderm-free area where ectoderm of the neural tube or plate and general surface ectoderm are in intimate contact. It is believed that this mesoderm-free area is in part produced by the withdrawal from the midline of prechordal mesoderm." In cases of cyclopia this withdrawal of prechordal mesoderm does not occur. Kingsbury and Adelmann conclude that this "might in some instances prevent the formation of a mesoderm-free area, and thus would account for the failure of hypophysis to form in so many cases of cyclopia." It may be that a mesoderm-free area is not produced in grafts from early donors, that therefore the skin ectoderm and nervous ectoderm fail to make contact and the hypophysis does not develop. The idea of contact suggests dependent differentiation for which the writer has previously found some experimental evidence (Stein, 1929).

However, another possibility may be considered. Hypophysis is chiefly absent from grafts of young donors, under eight somites, and where present occurs in very small amounts and only in the largest of the grafts. It is known that differentiation continues on the chorio-allantoic membrane while cell division is retarded (Hoadley, 1929). It may be that where cleavage was more retarded, in the smaller grafts, there was simply not enough material for a recognizable expression of the hypophysis. Cleavage is more retarded in small implants than in larger ones (Hoadley, 1929; Murray and Selby, 1930). This would account for the larger amount of hypophysis present in Willier and Rawles's grafts of total blastoderms of pre-somite stages and in Rudnick's grafts of the median area inclusive of the node of the definitive primitive-streak stage. It may be of interest to suggest here a possible cause of the inhibition of cleavage. Sorin (1928) has shown that the yolk undergoing liquefaction beneath the chick blastoderm in the first 4-5 days of incubation gives off rays capable of stimulating mitogenetic activity in plant cells. Tissues of the whole embryo in the early part of this period and of brain in the later part do not have this effect, but the blood of the embryo of 4-5 days has it. During implantation to the chorio-allantoic membrane implants are removed from yolk and do not get an

adequate blood supply for some hours (Henderson, 1930), and this happens at a time when cleavage is proceeding rapidly in the normal embryo. The absence of cleavage may therefore be due to the absence during a critical period of the agents normally stimulating it.

The degree of differentiation attained by the hypophysis is similar in all the grafts, regardless of the age of the donor. In grafts from younger implants fewer cords are formed and less hypophysial tissue is present. This naturally makes for a histological arrangement different from that in the normal embryo. The cords, being fewer in number, can scarcely fail to appear less anastomosing and to have fewer spaces filled with mesenchyme and blood capillaries.

LOCATION OF PRESUMPTIVE HYPOPHYSIAL MATERIAL

A series of grafts was made of anterior portions of head-process blastoderms, including varying amounts of the head-process, with the view of locating the material which later forms the hypophysis. Fifteen of these were examined in addition to the twenty-six of implants anterior to the head-process, and in all except one the hypophysis failed to differentiate. The one exception came from an implant limited anteriorly by the opaque area and extending posteriorly to include half of the head-process of a blastoderm in which the latter measured 0.6 mm. in length.

It had previously been found experimentally (Stein, 1929) that, in the eleven-somite stage, at least a part of the hypophysial material is located in a region of the ectoderm closely attached to the ventral surface of the forebrain and just anterior to the oral plate ectoderm. This is approximately the location given by Kingsbury (1922) from morphological studies. In a nine-somite embryo the "hypophysial area" constitutes the ventral skin ectoderm just posterior to the anterior neuropore. It may be mentioned that in two cases of embryos, one of eleven somites and one of seven somites, vitally stained by the writer anterior to Hensen's node in the definitive primitive-streak stage, the anterior neuropore and the material bordering on it were found blue upon examination. In the grafts made by Rudnick (1932) of central and lateral sections of the head-process and definitive primitive-streak stage the hypophysis, when present, was always in grafts from the central implant, which in-

cluded central material from the anterior limit of the pellucid area back to the posterior boundary of Hensen's node.

The foregoing data indicate that the presumptive hypophysial material is located in the mid-line anterior to the head-process or to Hensen's node in the respective stages.

SUMMARY AND CONCLUSIONS

1. This paper treats of the location and capacity for differentiation of the prospective forebrain and hypophysial ectoderm of the chick embryo. The method of study was by chorio-allantoic grafting.

2. The donors used for the study of the forebrain were of definitive primitive-streak, head-process, and early headfold stages. It was discovered that forebrain will almost invariably differentiate from anterior levels of the head-process stage excluding all head-process material. In a few cases it also differentiated from implants of the level anterior to Hensen's node in the definitive primitive-streak stage. If the levels lateral to the streak are included with the anterior level in the implant, differentiation of nervous material in the grafts is more frequent.

The results suggest that the forebrain is determined by the time the head-process has become morphologically indicated, that the time at which it becomes indicated is a critical period in the process of determination, but that a certain degree of determination may be present even in the definitive primitive-streak stage.

The experiments also indicate that material destined to give rise to forebrain lies anterior to the node in the definitive primitive-streak stage and anterior to the head-process in the latter stage.

3. Data from previous experiments in regard to the location and capacity for differentiation of the hypophysis and prospective hypophysial ectoderm have been summarized and additional data secured.

The capacity of this tissue for differentiation is extremely limited in implants of levels anterior to the node in headfold, head-process, and early somite embryos. It rises to about 55 per cent in implants from donors of eight to twelve somites. Above fifteen somites it is

considerably higher: 79 per cent from donors of fifteen to thirty somites, 94 per cent from donors of 3-5 days.

The low capacity of the hypophysis for differentiation from implants of early stages is considered. The possibilities suggested to account for it are the failure of the brain and skin ectoderm to make proper contact or the lack of sufficient cell division in the grafts.

The experiments reported here, together with those previously reported by the author and those of other investigators, indicate that in the definitive primitive-streak stage the prospective hypophysial ectoderm lies in the mid-line anterior to Hensen's node.

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